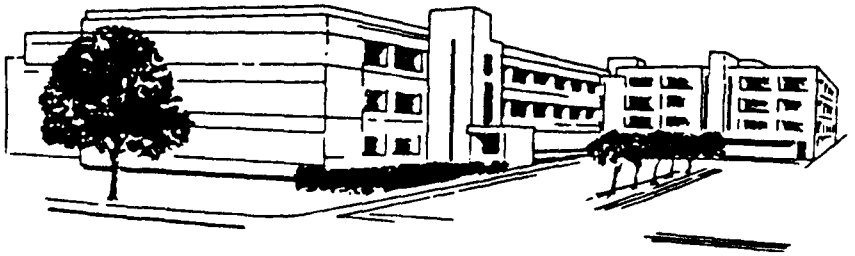
Laboratory Note No. 82

PYRIDOXINE DEFICIENCY AND WOUND HEALING IN RATS: ASSESSMENT OF ANTIOXIDANT ENZYMES AND IMMUNOLOGICAL STATUS

Thornton Samuel Mu
and
Michael A. Dubick

Division of Military Trauma Research

July 92



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

Pyridoxine Deficiency and Wound Healing in Rats: Assessment of Antioxidant Enzymes and Immunological Status -- Thornton S. Mu and Michael A. Dubick

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

The experimental studies of the author described in this report were reviewed and approved by the Institutional Review Committee/Animal Care and Use Committee at Letterman Army Institute of Research. The manuscript was peer reviewed for compliance prior to submission for publication. In conducting the research described here, the author adhered to the "Guide for the Care and Use of Laboratory Animals," DHEW Publication (NIH) 86-23.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

25 NOV 92

GEORGE J. BROWN (date)
COL, MC
Commanding

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT This document has been approved for public release, distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Laboratory Note No. 82	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Division of Military Trauma Research 6b. OFFICE SYMBOL (If applicable) SGRD-ULT-M	7a. NAME OF MONITORING ORGANIZATION U.S. Army Research and Development Command
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5012
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO.: 61102A PROJECT NO.: BS14 TASK NO.: S14/CC WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
(U) Pyridoxine Deficiency and Wound Healing in Rats: Assessment of Antioxidant Enzymes and Immunological Status

12. PERSONAL AUTHOR(S)
Thornton Samuel Mu and Michael A. Dubick

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Lab Notes	FROM _____ TO _____	July 92	14

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Pyridoxine, Wound healing, antioxidant, rats, immunoglobulins

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Many nutrients are involved in the complex process of wound healing. Of the vitamins, pyridoxine (vitamin B_6)deficiency has resulted in impaired healing, primarily due to its effect on amino acid and protein metabolism. This study examined the effects of pyridoxine deficiency on wound healing and its effects on acute phase proteins, immunoglobulins, and antioxidant enzymes in rats. Female rats were fed diets containing 0 mg/kg (deficient), 0.25 or 1.0 mg/kg (marginally deficient) or 7 mg/kg (control) pyridoxine for 5 weeks. Half the animals in each dietary group received a 3 cm long full skin incisional wound on the back and all animals were euthanized 1 week later. The results showed that despite pyridoxine deficiency, the animals in each dietary group had similar acute phase responses and antioxidant enzyme status in response to the injury.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL George J. Brown, COL, MC, Commanding	22b TELEPHONE (Include Area Code) (415) 561-5816	22c. OFFICE SYMBOL SGRD-ULT-M

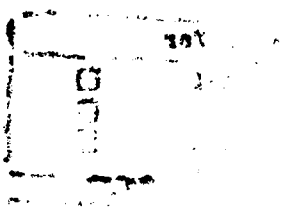
DD Form 1473, JUN 86 *Previous editions are obsolete.*

ABSTRACT

Many nutrients are involved in the complex process of wound healing. Of the vitamins, pyridoxine (vitamin B_6) deficiency has resulted in impaired healing, primarily due to its effect on amino acid and protein metabolism. This study examined the effects of pyridoxine deficiency on wound healing and its effects on acute phase proteins, immunoglobulins, and antioxidant enzymes in rats. Female rats were fed diets containing 0 mg/kg (deficient), 0.25 or 1.0 mg/kg (marginally deficient) or 7 mg/kg (control) pyridoxine for 5 weeks. Half the animals in each dietary group received a 3 cm long full skin incisional wound on the back and all animals were euthanized 1 week later. The results showed that despite pyridoxine deficiency, the animals in each dietary group had similar acute phase responses and antioxidant enzyme status in response to the injury.

FOREWORD

The work summarized in this report was part of an Inhouse Laboratory Independent Research project in the Division of Military Trauma Research. The work was performed by Mr. Mu during his tenure in the Division as part of the George Washington University-Department of Defense Science and Engineering Summer Apprenticeship Program for high school students.



PYRIDOXINE DEFICIENCY AND WOUND HEALING IN RATS: ASSESSMENT OF ANTIOXIDANT ENZYMES AND IMMUNOLOGICAL STATUS--Thornton Samuel Mu and Michael A. Dubick, Ph.D.

Wound healing is a complex process that takes place in many stages (1) and requires the presence of many important hormones, nutrients, and proteins, such as acute-phase proteins, immunoglobulins, and proteins and enzymes that regulate potentially damaging oxygen radicals (2-5). These oxygen radicals formed in response to trauma and tissue injury can cause serious cell injury, such as lipid peroxidation (6).

Among the nutrients, pyridoxine (vitamin B_6) plays an important role in amino acid and protein metabolism, including the synthesis and maturation of connective tissue proteins (7). Consequently, pyridoxine deficiency can affect normal wound healing (8). Pyridoxine also possesses an indirect antioxidant function that prevents damage done by oxygen radicals (9), functions as a coenzyme in the metabolism of amino acids (10), and is important for normal immune function (11). Because proteins, such as immunoglobulins, antioxidant enzymes, and acute-phase proteins, are composed of amino acids, it is assumed that a deficiency in vitamin B_6 would adversely affect production of these proteins.

An evaluation of combat casualties indicates that penetrating injuries account for up to 90% of all casualties (12). Although many of these are not life threatening, circumstances that impair the healing process can expend considerable military medical resources and delay the recovery of the injured soldier. Considering the potential importance of adequate pyridoxine nutriture to the healing process and the indication that about 50% of the U.S population consumes less than 70% of the RDA for vitamin B_6, a better understanding of the role of pyridoxine in wound healing could lead to improved therapeutic regimens or preventive measures, thus reducing morbidity and the consumption of medical resources by the injured patient.

We proposed, therefore, that pyridoxine deficiency would impair the ability to respond to injury. In this study we examined the effects of various stages of

pyridoxine deficiency on the status and presence (activity) of antioxidant enzymes and on the concentrations of certain acute-phase proteins and immunoglobulins in an incisional wound model in the rat.

MATERIALS and METHODS

Experimental protocol

Young adult, female Long-Evans rats, initially weighing 175-200 g were employed in these studies. A total of twenty-four rats were used in this phase of the study. The rats were randomly assigned to four groups of six rats. The first group was fed a diet devoid of (deficient) vitamin B_6. The second group was fed a diet with 0.25 mg vitamin B_6 per kg of diet. The third group was fed a diet with 1 mg/kg vitamin B_6. The fourth group was fed a control diet with 7 mg/kg vitamin B_6. All four groups were fed their respective diets for a period of five weeks. Three rats within each group underwent surgery in which an incision was made through the full thickness of the skin on the dorsal side. The rats recovered for one week after the surgery and were fed their respective diets during the recovery period.

Biochemical Studies

At the end of six weeks, the rats were overdosed with pentobarbital (120 mg/kg). Blood was withdrawn by cardiac puncture into citrated tubes and plasma was recovered by centrifugation for further evaluation of vitamin B_6 levels, immunoglobulin G and M, albumin, and fibrinogen. Liver, kidney, and brain were quickly removed, snap frozen in liquid nitrogen, and stored at -70°C in a biological freezer until assayed.

In preparation for assays to determine glutathione peroxidase, glutathione reductase, superoxide dismutase, and protein content, samples of kidney, liver, and brain were homogenized separately using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) in a homogenizing buffer composed of 0.25M sucrose and 10mM Tris-HCl, pH 7.4 for ten seconds. Using a Branson sonifier cell disruptor (Branson Ultra Sonic, Danbury, CT), the samples were sonicated three times

for a period of five seconds each. The samples were centrifuged for 30 minutes at 10,000 g in a RC-5 Superspeed Refrigerated Centrifuge (DuPont Instruments, Newton, CT).

The samples were tested for glutathione peroxidase by using hydrogen peroxide as a substrate (13) and tested for glutathione reductase activity (14) by measuring the disappearance of beta-nicotinamide adenine dinucleotide phosphate (beta-NADPH). Total superoxide dismutase (SOD) (13) activity was measured by the ability of the enzyme to inhibit the auto-oxidation of pyrogallol. Manganese SOD activity (13) was measured under the same conditions except the buffer contained 1mM KCN. CuZnSOD was calculated by subtracting MnSOD activity from the total SOD activity. All spectrophotometric assays used the Beckman DU-70 Spectrophotometer (Beckman Instruments, Fullerton, CA).

Immunoglobulin G and M (IgG and IgM, respectively) concentrations in plasma were determined in diffusion plates (Kallestad, Chaska, MN) by double immunodiffusion (15). The results were recorded after 24 and 48 hrs incubation but only the 48 hr values were used according to standard protocol in clinical laboratories. Ten µL of antibody was placed in the center well with dilutions of plasma placed in the outer wells. For IgM, neat through 1/32 dilutions were used and for IgG, 1/32 to 1/1024 dilutions were used.

Fibrinogen and albumin concentrations were determined by rocket immunoelectrophoresis (16) using the Bio-Rad horizontal electrophoresis cell (Bio-Rad Laboratories, Richmond, CA). Albumin concentrations were determined from standards, and fibrinogen concentrations were calculated as arbitrary units based on dilutions of normal rat plasma.

Protein content was determined with a commercially available kit (Bio-Rad, Richmond, CA) using the Beckman Du-70 spectrophotometer.

Statistical Analysis

Data were analyzed by two way ANOVA with injury and diet as factors. Significant differences were

further evaluated by the Newman-Keuls method for multiple comparisons. A $p<0.05$ was considered statistically significant.

Animal Use Statement

Animal quarantine and prior care were performed in accordance with SOP# OP-ARG-4 and OP-ARG-40 and conforms to the provisions of the NIH as stated in the Guide for the Care and Use of Laboratory Animals (NIH publication #85-23).

RESULTS

Pyridoxine status

Tests to determine pyridoxine status in rats showed that rats with no vitamin B_6 in their diets were indeed deficient in vitamin B_6 whereas the control rats with 7mg/kg of pyridoxine had normal vitamin B_6 concentrations in their plasma. The other two groups had intermediate vitamin B_6 levels in their plasma (data not shown).

Acute-phase proteins (Table 1)

Concentrations of fibrinogen were not significantly affected by either diet or injury whereas albumin concentrations were significantly lower in rats with injury ($p=0.0023$) and those fed pyridoxine deficient diets ($p=0.0002$).

Immunoglobulin G and M (Table 2)

Concentrations of IgG and IgM in plasma were not significantly different among the dietary groups. Plasma immunoglobulin concentrations in injured rats were also not significantly different from those in noninjured rats within each dietary group.

Antioxidant enzyme status (Table 3)

Activity of glutathione peroxidase per gram of tissue (GPx/g) was significantly higher ($p=0.0240$) in the livers of rats deficient in vitamin B_6 compared with controls. However, in brain and kidney samples,

GPx activity was not significantly affected by the different vitamin B_6 diets. In all dietary groups, GPx activity from brain samples from injured rats showed a significant decrease ($p=0.0229$) compared with their uninjured counterparts, whereas in their liver and kidney samples, a nonsignificant trend of decrease was noted.

In the rats that were deficient in vitamin B_6, activity of glutathione reductase per gram of tissue (GR/g) was significantly lower ($p=0.0278$) in liver samples, but significantly higher ($p=0.0034$) in brain samples compared with controls. Activity of GR/g in kidney was not significantly affected by diet or injury.

Differences in manganese superoxide dismutase activity per gram of tissue (MnSOD/g) were not significant among the diet and injury status of the four dietary groups in any of the tissues assayed.

However, activity of copper zinc superoxide dismutase per gram of tissue (CuZnSOD/g) was significantly lower ($p=0.0391$) in kidney samples from rats fed deficient pyridoxine diets, but was significantly higher ($p=0.0390$) in brain samples from rats fed the same deficient diets compared with controls. Liver sample activity was not significantly different among all dietary groups. In terms of injury status, CuZnSOD/g activity was not significantly affected in any of the three tissues assayed.

DISCUSSION

It has already been shown that vitamin B_6 affects protein metabolism (10), and in our studies, protein levels of rats with diets deficient in vitamin B_6 were significantly reduced (data not shown). However, similar to studies by Schaeffer et al. (17), body weight of all rats remained relatively constant despite the varying amount of pyridoxine in their diets (data not shown). Therefore, to insure more stable readings, activity of the antioxidant enzymes measured were expressed per grams of tissue rather than grams of protein.

Our results have shown that there were few significant decreases in enzyme activity due to changes in pyridoxine diet or injury status. One could possibly assume that with a shortfall of protein, amounts of other proteins and enzymes could also decrease. Yet this assumption did not hold for the enzymes and proteins measured in our studies because fibrinogen, IgG, IgM, GPx, GR, MnSOD, and CuZnSOD concentrations did not decrease significantly. Instead, in tissues in which antioxidant enzyme activity was significantly affected by diet, the values were usually higher in the deficient group than in the controls.

One possible explanation for these findings is that the one week allowed for recovery from surgery may have been enough time to synthesize the enzymes and proteins listed above. In addition, a previous study by Black et al. showed that rats with pyridoxine-deficient diets maintained a vitamin B_6 reserve in their gastrocnemius muscle (18). Therefore, although the plasma revealed a pyridoxine deficiency, the rats may have been relying on this storage to provide tissues with enough pyridoxine to help synthesize the needed enzymes and proteins to respond to the stress of the injury induced.

Albumin levels, on the other hand, showed a significant decrease both in the vitamin B_6 group and the injured group. Albumin in plasma was affected by pyridoxine deficiency, but the reaction of albumin as an acute-phase protein following injury was not impaired.

In conclusion, pyridoxine deficiency did not significantly affect the rat's response to surgically-induced wound. The antioxidant status of the tissues examined in the rats remained at control levels or higher, and the immunoglobulins seemed to be unaffected by the pyridoxine deficiency. Also, the acute-phase protein response to injury was not adversely affected by the pyridoxine deficiency. More research is needed to gain a better understanding of the effects of vitamin B_6 in the realm of wound healing.

Acknowledgements

The authors would like to thank Dr. Virginia Gildengorin for assisting with the statistical analyses; Ms. Susan Siefert for helping me prepare this paper; and the Military Trauma Research Division and COL Brown, MAJ McCollum, and Mr. William Ward of the Command Division at the Letterman Army Institute of Research for giving me (TSM) this tremendous opportunity. The authors also thank SPC Jacqueline Y. Greene for animal husbandry, Ms. Denise Gretz of the Agricultural Research Service, USDA for the pyridoxine assays, and Mr. Donald L. Calkins for preparation of this report.

REFERENCES

1. Hotter, AN. Physiologic aspects and clinical implications of wound healing. Heart Lung 1982;11:522-530.

2. Kushner, I. The Acute Phase Response: An Overview. Methods in Enzymology 1988;163:373-383.

3. Ritzman SE, Daniels JC. Laboratory Notes: Serum Proteins, Somerville, NJ: Behring Diagnostics, Nov. 1-28, 1973.

4. Murphy FA, Osebold JW, Aalund O. Kinetics of the antibody response to Anaplasma marginale infection. J Infect Dis 1966;16:99.

5. Harris ED. Regulation of antioxidant enzymes. FASEB J 1992;6:2675-2683.

6. Slater TF. Free-radical mechanisms in tissue injury. Biochem J 1984;222:1-15.

7. Tinker D, Rucker RB. Role of selected nutrients in synthesis, accumulation, and chemical modification of connective tissue protein. Physiol Rev 1985;65:607-657.

8. Lakshmi AV, Diwan PV, Bamji MS. Wound healing in pyridoxine deficiency. Nutr Res 1988;8:1203-1206.

9. Wills ED. The role of dietary components in oxidative stress in tissues. In: Oxidative Stress, H Sies, ed., Academic Press, New York, 1985, pp. 197-218.

10. Dubick MA. Interactions of vitamin B_6 and xenobiotics. In: Nutritional Toxicology, Hathcock JN, ed., Academic Press, Orlando, 1989;3:97-121.

11. Chandra RK, Sudhakaran L. Regulation of immune responses by vitamin B_6. In: Vitamin B_6, Dakshinamurti K, ed., Academic Press, New York, 1990, pp. 404-423.

12. Bellamy RF, Zajtchuk R. The management of ballistic wounds of soft tissue. In: Textbook of Military Medicine, v.5, Zajtchuck R, Jenkins, DP, Bellamy RF, Quick CM, eds., US Government Printing Office, Washington, D.C., 1991, pp.163-220.

13. Zidenberg-Cherr S, Han B, Dubick MA, Keen CL. Influence of dietary-induced copper and manganese deficiency on ozone-induced changes in lung and liver antioxidant systems. Toxicol Lett 1991;57:81-90.

14. Cohen MB, Duvel DL. Characterization of the inhibition of glutathione reductase and the recovery of enzyme activity in exponentially growing murine leukemia (L1210) cells treated with 1,3-bis(2-chloroethyl)-1-nitrosourea. Biochem Pharm 1988;37:3317-3320.

15. Clausen J. Immunochemical techniques for the identification and estimation of macro-molecules. New York: American Elsevier Publishing Company, Inc., 1969.

16. Laurell, CB. Electroimmunoassay. Scand J Clin Lab Invest 1972;29(suppl. 124):21-37.

17. Schaeffer MC, Sampson DA, Skala JH, Gietzen DW, Grier RE. Evaluation of vitamin B_6 status and function of rats fed excess pyridoxine. J Nutr 1989;119:1392-1398.

18. Black AL, Guirard BM, Snell EE. The behavior of muscle phosphorylase as a reservoir for vitamin B_6 in the rat. J Nutr 1978;108:670-677.

TABLE 1

Effects of Vitamin B_6 Status and Injury on Plasma Acute Phase Protein Concentrations[1]

Vitamin B_6		Fibrinogen[2]	Albumin[3,4]
0 mg/kg	Uninjured	6.2±3.2	5.8±0.5
	Injured	11.2±1.8	4.8±0.3
0.25 mg/kg	Uninjured	13.4±2.1	5.6±0.2
	Injured	9.1±3.3	4.8±0.2
1.0 mg/kg	Uninjured	11.0±1.6	6.8±0.4
	Injured	12.4±0.6	6.8±0.6
7.0 mg/kg	Uninjured	10.5±1.6	7.9±0.2
	Injured	10.1±2.5	5.9±0.1

[1]Data expressed as mean ±S.E. (n=3)
[2]Values represent arbitrary units/dl
[3]Values represent g/dl
[4]Significant effect of diet and injury at $p<0.05$

TABLE 2

Summary of Plasma Immunoglobulin Titers in Injured and Uninjured Rats fed Pyridoxine-deficent diets.

Diet		IgG	IgM
0 mg	Uninjured	1:128	1:4
	Injured	1.128	1:4
0.25 mg	Uninjured	1:256	1:4
	Injured	1:256	1:4
1.0 mg	Uninjured	1:128	1:4
	Injured	1:128	1:2
7.0 mg	Uninjured	1:128	1:4
	Injured	1:128	1:4

TABLE 3

Effect of Vitamin B_6 Status and Injury on Tissue Antioxidant Enzymes[1]

		Vitamin B_6 Diet			
		0 mg/kg	0.25 mg/kg	1.0 mg/kg	7.0 mg/kg
Glutathione Peroxidase					
Liver[3]	Uninjured	105.6±9.9	82.6±4.0	100.4±15.9	89.3±2.3
	Injured	99.9±10.0	67.0±5.7	87.9±3.7	85.2±5.2
Kidney	Uninjured	11.7±0.7	13.4±0.9	16.0±1.4	14.7±0.8
	Injured	14.1±1.1	10.4±2.0	14.0±0.9	13.5±0.5
Brain[4]	Uninjured	0.92±0.01	0.80±0.06	0.88±0.05	0.88±0.14
	Injured	0.85±0.06	0.72±0.02	0.83±0.04	0.64±0.08
Glutathione Reductase					
Liver[3]	Uninjured	11.2±1.0	9.7±0.4	10.1±0.9	12.5±1.4
	Injured	8.3±0.05	9.2±2.1	11.9±0.3	12.4±3.7
Kidney	Uninjured	12.8±0.7	11.3±1.5	13.1±1.2	13.1±1.1
	Injured	12.5±0.5	9.2±2.1	11.9±0.3	12.4±3.7
Brain[3]	Uninjured	1.09±0.13	1.21±0.01	1.42±0.07	0.65±0.21
	Injured	1.34±0.04	1.30±0.08	1.35±0.08	0.97±0.25

TABLE 3 Continued

Mn SOD[2]					
Liver	Uninjured	532±109	735±154	875±79	840±204
	Injured	766±234	585±38	540±38	692±70
Kidney	Uninjured	436±12	472±34	390±101	408±28
	Injured	559±53	312±47	401±75	433±56
Brain	Uninjured	131±3	142±3	139±15	145±21
	Injured	129±12	150±14	127±11	118±9
CuZn SOD[2]					
Liver	Uninjured	5480±791	4935±1296	4578±1001	4428±43
	Injured	4996±669	3598±702	5442±237	4975±220
Kidney[3]	Uninjured	1075±40	1118±250	1706±177	1146±126
	Injured	1071±207	1398±308	1548±88	1255±19
Brain[3]	Uninjured	437±16	365±37	348±46	371±26
	Injured	469±16	386±0.3	323±67	349±50

[1]Date expressed as mean ±S.E. (n=3)
[2]Superoxide dismutase
[3]Significant effect of diet at $p<0.05$
[4]Significant effect of injury at $p<0.05$

14-- Mu

'OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
& Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, MD 21701-5012

Defense Technical Information Center
ATTN: DTIC/DDAB (2 copies)
Cameron Station
Alexandria, VA 22304-6145

Office of Under Secretary of Defense
Research and Engineering
ATTN: R&AT (E&LS), Room 3D129
The Pentagon
Washington, DC 20301-3080

DASG-AAFJML
Army/Air Force Joint Medical Library
Offices of the Surgeons General
5109 Leesburg Pike, Room 670
Falls Church, VA 22041-3258

HQ DA (DASG-ZXA)
WASH DC 20310-2300

Commandant
Academy of Health Sciences
US Army
ATTN: HSHA-CDM
Fort Sam Houston, TX 78234-6100

Uniformed Services University of
Health Sciences
Office of Grants Management
4301 Jones Bridge Road
Bethesda, MD 20814-4799

US Army Research Office
ATTN: Chemical and Biological
Sciences Division
PO Box 12211
Research Triangle Park, NC 27709-2211

Director
ATTN: SGRD-UWZ-L
Walter Reed Army Institute of Research
Washington, DC. 20307-5100

Commander
US Army Medical Research Institute
of Infectious Diseases
ATTN: SGRD-ULZ-A
Fort Detrick, MD 21701-5011

Commander
US Army Medical Bioengineering Research
and Development Laboratory
ATTN: SGRD-UBG-M
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Medical Bioengineering
Research & Development Laboratory
ATTN: Library
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Research Institute
of Environmental Medicine
ATTN: SGRD-UE-RSA
Kansas Street
Natick, MA 01760-5007

Commander
US Army Research Institute of
Surgical Research
Fort Sam Houston, TX 78234-6200

Commander
US Army Research Institute of
Chemical Defense
ATTN: SGRD-UV-AJ
Aberdeen Proving Ground, MD 21010-5425

Commander
US Army Aeromedical Research
Laboratory
Fort Rucker, AL 36362-5000

AIR FORCE Office of Scientific
Research (NL)
Building 410, Room A217
Bolling Air Force Base, DC 20332-6448

USAF School of Aerospace Medicine
Document Section
USAFSAM/TSKD
Brooks Air Force Base, TX 78235-5301

Head, Biological Sciences Division
OFFICE OF NAVAL RESEARCH
800 North Quincy Street
Arlington, VA 22217-5000

Commander
Naval Medical Command-02
Department of the Navy
Washington, DC 20372-5120